Supplemental materials

Methods

Pharmacokinetic assessment

Nonclinical

Plasma samples (50 µL) were mixed with 0.5% formic acid aqueous solution (200 µL), methanol (10 µL), NaOH solution (MeOH:water:1-mol/L NaOH (80:19:1), 10 µL), and internal standard solution (20 µL). The mixture was loaded on an OASIS HLB µElution 96-well plate (30 µm, Waters Corporation), washed with 0.5% formic acid aqueous solution and eluted with 100 µL of methanol. The eluate (60 µL) was mixed with 0.5% formic acid aqueous solution (60 µL), and the resulting solution (10 µL) was injected into the liquid chromatography-tandem mass spectrometry (LC-MS/MS) system.

Ten-fold diluted heart homogenate samples (100 µL) were mixed with acetonitrile (100 µL), methanol (10 µL), NaOH solution (MeOH:water:1-mol/L NaOH (80:19:1), 10 µL), and internal standard solution (20 µL). Then the mixture was centrifuged (12,000 rpm, 2 min, 10°C). The supernatant (100 µL) was mixed with 0.5% formic acid aqueous solution (100 µL) and the mixture was loaded on an OASIS HLB µElution 96-well plate (30 µm, Waters Corporation), washed with 0.5% formic acid aqueous solution and eluted with 100 µL of methanol. The eluate (60 µL) was mixed with 0.5% formic acid aqueous solution (60 µL), and the resulting solution (20 µL) was injected into the LC-MS/MS system.

XBridge C18 column (3.5 µm, 2.1 × 50 mm, Waters Corporation) was used. Mobile phase A was 0.05% formic acid aqueous solution, and mobile phase B was methanol containing 0.05% formic acid. Mobile phase program was isocratic flow (0.3 mL/min) of mobile phase A/B (3/7). The retention times of amiselimod, amiselimod-P, fingolimod and fingolimod-P were 2.5, 2.4, 3.5 and 3.2 min, respectively.

Clinical

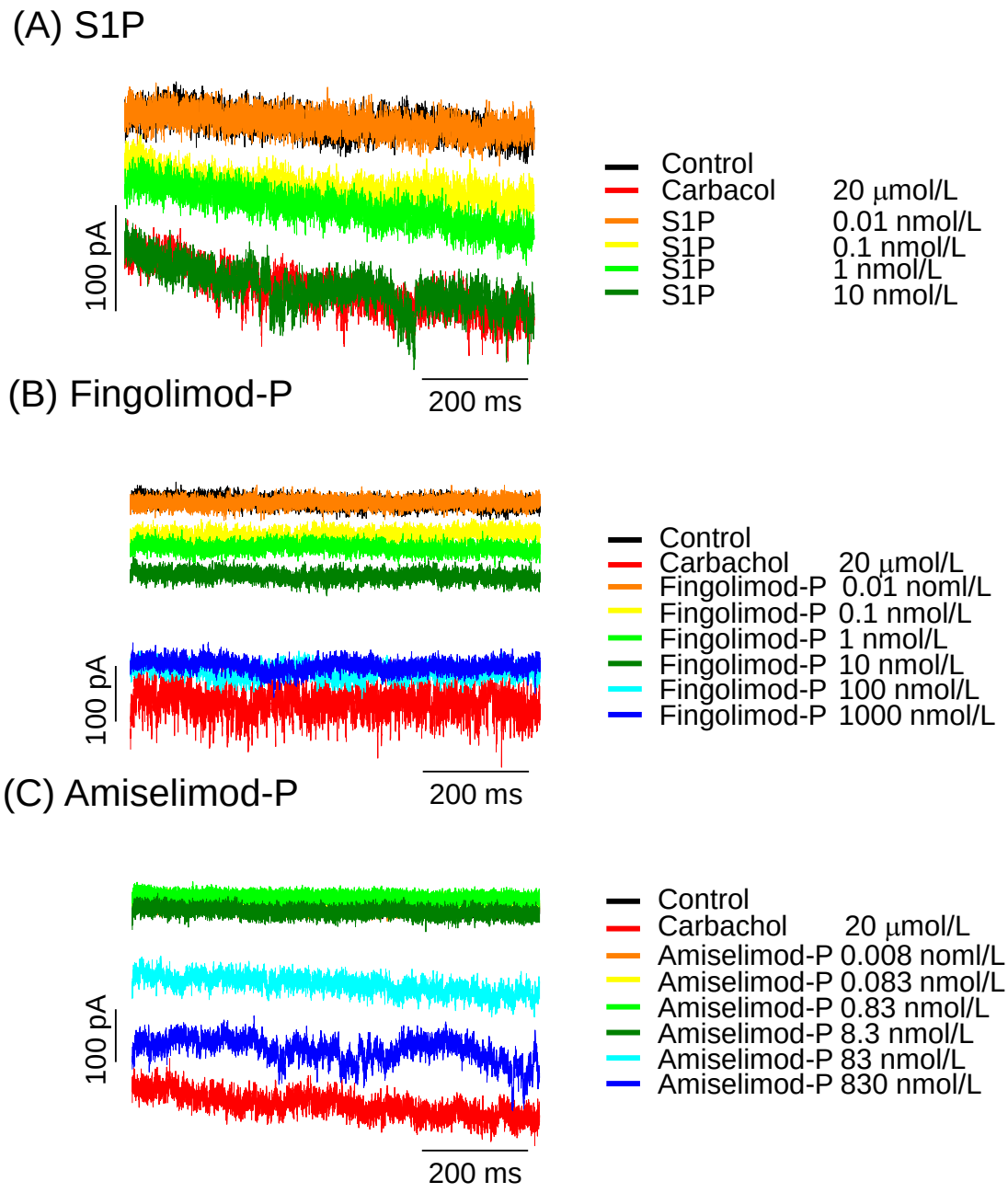
The concentrations of plasma amiselimod and amiselimod-P were measured using a fully validated LC/MS/MS.

For determination of amiselimod and amiselimod-P, blood samples were collected into polypropylene tubes containing dipotassium-ethylenediamine-tetra acetic acid (K2-EDTA) at each scheduled time-point. Samples were centrifuged at 1500 g for 10 minutes at 4°C within 30 minutes after collection to obtain plasma. These plasma samples were extracted with a solid phase extraction cartridge, OASIS HLB (1 cc/10 mg, Waters Corporation). To the eluate, 0.5% formic acid was added, mixed and

injected into the LC/MS/MS system.

For amiselimod, LC/MS/MS was equipped with an L-column ODS (2.1 mm I.D. × 50 mm, particle size 5 μm, Chemical Evaluation and Research Institute) column, using a gradient elution with 10 mmol/L formic acid solution and methanol containing 10 mmol/L formic acid as the mobile phase. Amiselimod was detected by multiple reaction monitoring (MRM) mode with positive ion (*m/z* 378 → 175).

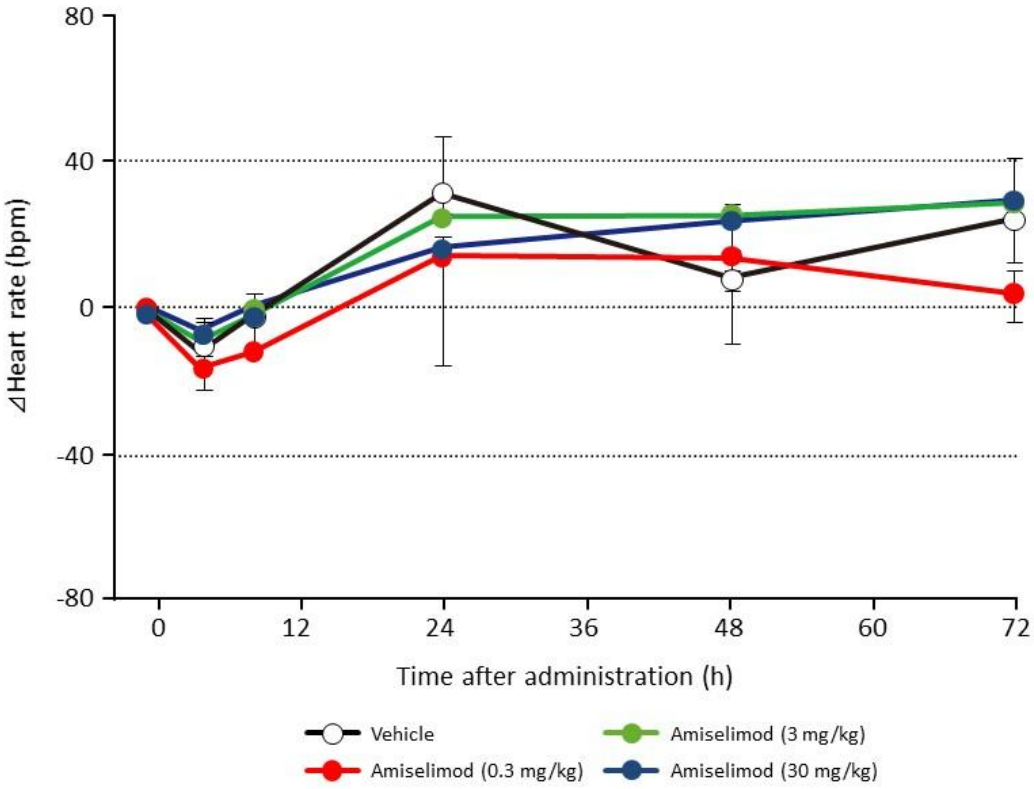
For amiselimod-P, a Gemini-NX (2.00 mm I.D. × 50 mm, particle size 3 μm, Phenomenex) column was equipped in LC/MS/MS, using a gradient elution with 0.056% ammonia solution and methanol containing 0.056% ammonia as the mobile phase. Amiselimod-P was detected by MRM mode with positive ion (*m/z* 456 → 79).



Supplemental Figure 1

Effects of amiselimod-P, fingolimod-P, and S1P on human GIRK currents

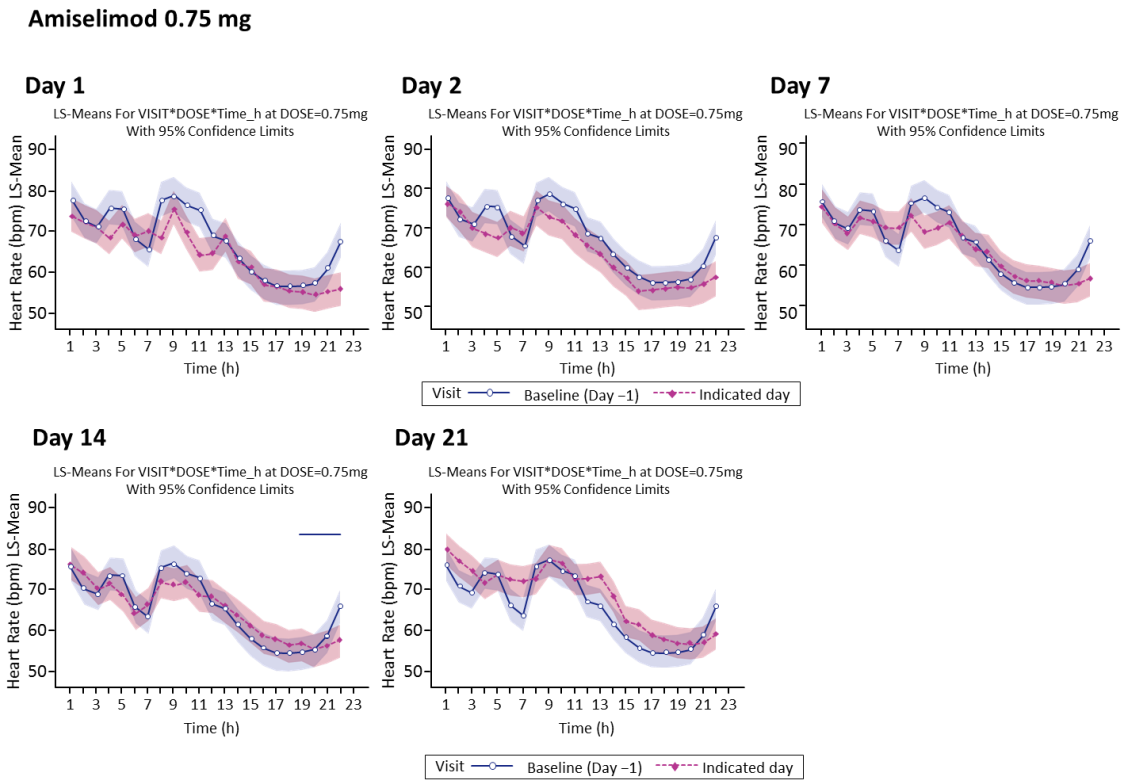
GIRK currents recorded in human atrial myocytes were measured using the whole-cell patch clamp method. Representative trace at −100 mV illustrating the effects of S1P (A), fingolimod-P (B), and amiselimod-P (C) on GIRK currents.



Supplemental Figure 2

Effect of amiselimod on heart rate in cynomolgus monkeys

Amiselimod was orally administered to conscious male cynomolgus monkeys in a dose-ascending manner at dose levels of vehicle, 0.3, 3 and 30 mg/kg, with a 6-day interval between vehicle and 0.3 mg/kg, and 13- or 14-day intervals between dose levels of 0.3, 3 and 30 mg/kg. Heart rate and ECG were analysed before and at 4, 8, 24, 48 and 72 h after each dose using the telemetry system. Results are expressed as the mean ± SD (n=4).



Supplemental Figure 3

Effects of 0.75 mg of amiselimod on mean hourly heart rate in healthy subjects.

Mean hourly heart rate was measured by 24-hour 12-lead Holter ECG. Curves on day 1 to day 21 are shown as pink bands, and the curve on day −1 as the blue band. Data were analysed using the linear mixed effect model and 95% confidence intervals are shown as the shaded area.

Supplemental Table 1

Pharmacokinetic parameters of amiselimod/amiselimod-P or fingolimod/fingolimod-P after a single oral administration of amiselimod or fingolimod to rats

Analyte	Tissue	t_{max} (h)	t_{last} (h)	C_{max} (ng/mL or g)	$AUC_{0\text{-}last}$ (ng·h/mL or g)
Amiselimod	Heart	8	48	174.2	3358
Amiselimod-P	Heart	8	48	42.21	846.2
Amiselimod	Plasma	2	8	4.072	23.85
Amiselimod-P	Plasma	2	48	31.26	524.7
Fingolimod	Heart	8	48	416.0	11444
Fingolimod-P	Heart	8	48	478.7	17182
Fingolimod	Plasma	8	24	4.192	68.45
Fingolimod-P	Plasma	8	48	21.45	556.0

Supplemental Table 2

Supplemental Table 2a. Summary of amiselimod derived pharmacokinetic parameters on day 1 and day 21

	PK parameter		Amiselimod (mg)			
			0.125 mg ** (n=10)	**0.25 mg (n=10)**	**0.5 mg (n=10)**	**0.75 mg (n=10)**
Day 1	**C_{max} (ng/mL)**	**Mean**	0.0441	0.1498	0.2473	0.3369
		SD	0.0314	0.0322	0.0482	0.0564
	t_{max} (h)	**Median (Min–Max)**	16.00 (12.00–23.98)	12.00 (8.00–23.97)	12.00 (12.00–23.97)	12.00 (12.00–24.00)
	AUC_{τ} (ng.h/mL)	**Mean**	0.525	2.592	4.364	5.986
		SD	0.463	0.573	0.812	1.128
	Ae_{0-24} (%)	**Mean**	0.0000	0.0009	0.0033	0.0086
		SD	0.0000	0.0029	0.0059	0.0057
	CL_R (L/h)	**Mean**	0.0000	0.00067	0.00349	0.01192
		SD	0.0000	0.00213	0.00603	0.00939

	PK parameter		Amiselimod (mg)			
			0.125 mg (n=10)	**0.25 mg (n=10)**	**0.5 mg (n=10)**	**0.75 mg (n=10)**
Day 21	**C_{max} (ng/mL)**	**Mean**	0.8792	1.8724	3.5585	4.6838
		SD	0.1841	0.2509	0.4528	0.8138
	t_{max} (h)	**Median (Min-Max)**	10.00 (1.00–12.00)	10.00 (8.00–12.02)	6.00 (1.00–16.00)	10.00 (2.00–12.00)
	AUC_{τ} (ng.h/mL)	**Mean**	17.514	39.722	73.606	95.710
		SD	3.057	5.658	7.392	15.055
	$t_{½}$ (h)	**Mean**	415	409	386	423
		SD	72	63	55	54
	$Ae_{480-504}$ (%)	**Mean**	0.0600	0.1107	0.1263	0.1513
		SD	0.0581	0.0521	0.0740	0.0659
	CL_R (L/h)	**Mean**	0.00436	0.00680	0.00860	0.01186
		SD	0.00422	0.00282	0.00495	0.00478
	Accumulation ratio	**Mean**	28.70	15.71	17.22	16.41
		SD	9.87	2.34	2.50	3.41

** n=7 for t_{max}

Supplemental Table 2b. Summary of amiselimod-P derived pharmacokinetic parameters on day 1 and day 21

	PK parameter		Amiselimod (mg)			
			0.125 mg (n=10)	**0.25 mg (n=10)**	**0.5 mg (n=10)**	**0.75 mg (n=10)**
Day 1	**C_{max} (ng/mL)**	**Mean**	0.3101	0.7224	1.1947	1.7566
		SD	0.0481	0.2335	0.2671	0.2906
	t_{max} (h)	**Median (Min–Max)**	12.00 (12.00–12.02)	12.00 (8.00–12.00)	12.00 (8.00–12.00)	12.00 (8.00–12.00)
	AUC_{τ} (ng.h/mL)	**Mean**	4.523	10.910	17.293	25.610
		SD	0.714	3.237	3.093	4.105
	$Ae_{0–24}$ (%)	**Mean**	0.0000	0.0000	0.0000	0.0000
		SD	0.0000	0.0000	0.0000	0.0000
	CL_R (L/h)	**Mean**	0.0000	0.0000	0.0000	0.0000
		SD	0.0000	0.0000	0.0000	0.0000

	PK Parameter		Amiselimod (mg)			
			0.125 mg (n=10)	**0.25 mg (n=10)**	**0.5 mg (n=10)**	**0.75 mg (n=10)**
Day 21	**C_{max} (ng/mL)**	**Mean**	1.4265	3.7619	7.2790	9.2227
		SD	0.1926	0.7458	1.6729	1.5565
	t_{max} (h)	**Median (Min–Max)**	8.00 (8.00–12.00)	8.01 (8.00–12.02)	8.00 (4.00–12.00)	12.00 (8.00–12.00)
	AUC_{τ} (ng.h/mL)	**Mean**	29.990	80.217	154.359	195.065
		SD	4.643	15.655	33.975	33.795
	$t_{½}$ (h)	**Mean**	378	404	376	385
		SD	65	61	41	52
	$Ae_{480–504}$ (%)	**Mean**	0.0091	0.0441	0.0353	0.0326
		SD	0.0150	0.0156	0.0172	0.0079
	CL_R (L/h)	**Mean**	0.00044	0.00178	0.00140	0.00153
		SD	0.00071	0.00096	0.00068	0.00031
	Accumulation ratio	**Mean**	6.70	7.57	9.05	7.78
		SD	1.02	1.08	1.77	1.68